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Commentary

Interphase Cytogenetics and Its Role in Molecular Diagnostics of Solid Tumors

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Interphase cytogenetics refers to the visualization of chromosomal aberrations in intact cell nuclei based on *in situ* hybridization with labeled nucleic acid probes. The concept of interphase cytogenetics was introduced in 1986 when Cremer and colleagues succeeded in scoring copy numbers of chromosome 18 in nuclei from normal cells and in nuclei from cells with a trisomy for chromosome 18 after probing the nondividing cells with centromere-specific DNA.[1] The enumeration of chromosomal copy numbers was soon extended to the visualization of structural chromosomal aberrations as well using either whole chromosome painting probes[2] or probes that specifically target known chromosomal rearrangements.[3,4] The identification of both numerical and structural chromosomal aberrations in the interphase cell nucleus is conceptually intriguing because it allows for the simultaneous assessment of chromosomal aberrations, cellular phenotype, and tissue morphology. Interphase cytogenetics can be applied to paraffin-embedded, formalin-fixed tissue sections, thus enabling retrospective analyses and correlation of chromosome alterations with biological and clinical end points. In addition, clonal heterogeneity can be readily identified. Interphase cytogenetics in solid tumor analyses was used for the identification of chromosomal copy number changes, eg, at the adenoma-carcinoma sequence during colorectal carcinogenesis,[5,6] in bladder carcinomas,[7] in cancers of the head and neck,[8] in lung carcinomas,[9] and in germ cell tumors.[10] A recent review by van Dekken et al[11] summarizes the data for some epithelial cancers.

The article in this issue by Bulten and colleagues[12] describes the use of *in situ* hybridization with selected centromere-specific repeat probes for the enumeration of chromosomal copy numbers directly in tissue sections of preinvasive lesions and invasive carcinomas of the uterine cervix. The study is aimed at the identification of specific chromosomal changes that correlate with disease progression. Several conclusions become possible. First, chromosomal aneusomy can be detected in all high-grade and persisting cervical intraepithelial neoplasias (CINs) and in all invasive carcinomas. Second, clearly, the number of chromosomal aberrations increases with tumor progression and, of note, does so in a nonrandom fashion. Some chromosomal markers, specifically chromosomes 1 and 7 and the X chromosome seem to be correlated with the progression from low-grade CIN lesions to high-grade lesions and invasive carcinomas. Therefore, these markers may evolve as objective predictors of tumor progression. Third, in addition to the mere identification of nonrandom chromosomal aberrations, the analysis of tissue sections has offered insight into the spatial distribution of cells carrying abnormal chromosomes. Indeed, the present study describes foci of aberrant cell clones in dysplastic cervical lesions, meaning that only a subset of the cells carry specific genetic alterations. It is likely that these foci are the seed for the diffuse aneusomy observed in invasive carcinomas and that the detection of these foci bears prognostic significance.

The paper by Bulten and colleagues[12] exemplifies one of the great advantages of interphase cytogenetics, ie, the possibility to investigate genetic aberrations in the context of and together with histomorphological features. This property alone underlines the significance of interphase cytogenetics for diagnosing cancer. More importantly, however, interphase cytogenetics actually allows for the sensitive detection and precise enumeration of the cell pool that carries specific chromosomal abnormalities. The high sensitivity seems to be particularly useful for the identification of chromosomal aberrations in precancerous lesions and thus for the characterization and description of early, possibly disease-initiating, chromosomal changes. These early chromosomal aberrations in a small percentage of cells are difficult to track down and are likely to escape detection if techniques that rely on the use of extracted tumor DNA, such as polymerase chain reaction or comparative genomic hybridization (CGH)[13,14] would be used exclusively. Due to the unavoidable dilution of cancerous cells with nonaberrant adjacent epithelial cells and stromal and inflammatory contamination, this becomes an important issue in solid

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tumor analyses. In addition, these methodologies do not preserve tissue morphology. Interphase cytogenetics, however, is an almost ideally suited methodology to identify chromosomal aberrations on a cell-to-cell level. In this respect, it will be a rewarding task to thoroughly evaluate the prognostic power of interphase cytogenetics. It is quite conceivable that the identification of subpopulations of aberrant cell clones, either in tissue sections or in cytological preparations, develops into sound prognostic indicators for the progression potential of premalignant lesions and will likely carry weight on treatment decisions.

The diagnostic challenge will shift from the confirmation of more or less advanced invasive cancers to the meaningful classification of early neoplastic lesions. Preventive screening programs, increased awareness and compliance, and improved detection technologies will undoubtedly contribute to this shift. The need for a precise forecast of the growth behavior of early and small lesions will pose difficulties for cytomorphology-based diagnostics, because the plethora of phenotypic consequences of genetic aberrations acquired during disease progression is less obvious at early stages. With that said, it becomes clear that the need to combine genetic and chromosomal markers with morphology will increase.

One of the limitations of the approach used by Bulten and colleagues[12] is the exclusive use of centromere-specific probe sets for the enumeration of chromosomal copy number changes. The obvious drawback is the selectivity of the approach as only a subset of chromosomes can be practically analyzed. Second, in a strict cytogenetic sense, centromere-specific probes can only be used to score centromere copy numbers. Although certainly useful for the enumeration of chromosomal aberrations such as monosomies or trisomies of a given chromosome, copy number changes affecting only chromosomal arms remain elusive. For instance, the formation of isochromosomes (ie, chromosomes that contain both arms of a normal chromosome, resulting in a trisomy for one arm that is accompanied by a monosomy of the other arm) cannot be identified by the interphase cytogenetic approach used by Bulten et al.[12] And isochromosomes are common themes in solid tumors.[15] Furthermore, chromosomal copy number changes that affect only one or a few chromosomal bands would escape the detection. Such subchromosomal copy number changes, however, are frequently observed in solid tumors and present themselves on the cytogenetic level as deletions, duplications, and homogeneously staining regions or double minute chromosomes.[15] The value of interphase cytogenetic analyses of tumor sections could therefore be greatly enhanced if locus- and gene-specific probes that target recurring, tumor-specific aberrations, such as isochromosomes, deletions, and gene amplifications, would be included in the analyses.

How to best achieve this goal? The recently developed technique of comparative genomic hybridization[13,14] and its application to the study of recurring chromosomal aberrations in solid tumors has added significantly to our knowledge of such specific chromosomal markers (reviewed, eg, by Forozan et al[16] and Ried et al[17]). CGH is a genome-scanning techniques that allows one to identify and map chromosomal and subchromosomal gains and losses. Virtually all papers published in the course of the last few years could identify a tumor-specific pattern of chromosomal copy number changes, hence a blueprint of chromosomal aberrations. One of the major advantages of CGH is its applicability to the retrospective study of archival tumor specimens.[18,19] Most importantly, CGH can be applied to establish the correlation of the tumor phenotype with chromosomal changes and to delineate the sequence of chromosomal aberrations as they occur in solid tumor progression if CGH is performed with DNA extracted from histologically defined and microdissected tumor material.[20–22] The application of the emerging knowledge of tumor- and tumor-stage-specific aberrations to complement cytomorphology-based classification of tumor stages will become a major task in cytopathology. The tool to translate this knowledge will clearly not be CGH. Interphase cytogenetics with chromosome- and gene-specific probes based on CGH results offers an elegant, simple, and fast procedure to move basic knowledge from bench to bedside. Precise diagnosis and staging, however, will certainly require a multiparameter analysis that will have to combine the assessment of recurring chromosomal aberrations and the status of pertinent tumor suppressor genes and oncogenes. The technical challenges here remain on how to develop dependable tests that allow the simultaneous identification of multiple markers. In this respect, the pioneering work by Hopman and colleagues[23] regarding multiparameter *in situ* hybridization for bright-field detection opens new avenues, and methodologies to increase the sensitivity of *in situ* hybridization will likely allow one to routinely detect even single-copy probes in interphase cells.[24,25] In addition, optical scanning or deconvolution techniques would facilitate the interpretation of signals in tissue sections, and the development and improvement of integrated microscope hardware and image analysis software will help to investigate large tumor cell populations. Another considerable advantage of interphase cytogenetics is provided by the possibility to combine chromosomal aberrations not only with the histological phenotype but also with pertinent immunophenotypic markers.[26,27]

To conclude, recently developed molecular cytogenetic screening tests for copy number changes in tumor genomes, in particular, CGH,[13,14] are ideal tools to screen tumor DNA for genomic imbalances and have been successfully applied to identify recurrent chromosomal aberrations in invasive carcinomas and their precursor lesions. Together with data regarding the status of specific oncogenes or tumor suppressor genes, and high-resolution gene expression profiling techniques,[28,29] the knowledge on tumor-specific genetic changes will expand. Accordingly, the value of interphase cytogenetics for the initial screening for chromosomal aberrations will decrease. However, interphase cytogenetics will become an increasingly important method to detect tumor-specific genetic aberrations in cytological preparations and tissue sections and to visualize aberrant cell clones in early lesions, as exemplified by Bulten and colleagues.[12] This will facilitate the trans-

lation of basic research to clinical practice. Such a translation will benefit from better understanding of tumor-stage-specific genetic alterations, from clinical trials that attempt to correlate such aberrations with the course of the disease, from the availability of suitable DNA clones for recurrently involved chromosomal regions or genes, and lastly from methodological developments regarding *in situ* hybridization cytochemistry and imaging tools for multiple target visualization of genetic and phenotypic markers. Eventually, one would hope that emerging data would be connected to and catalogued in the frame of the cancer genome anatomy project[30] to ensure cross-linking with genetic and clinical databases. Provided parallel progress in these fields, interphase cytogenetics may very well become one of the most valuable tools to integrate cytomorphology and genetics in the diagnostic laboratory.

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